Letter to the Editor

AFLP Analysis of a Collection of Tetraploid Wheats Indicates the Origin of Emmer and Hard Wheat Domestication in Southeast Turkey

H. Özkan, A. Brandolini,† R. Schäfer-Pregl,‡ and F. Salamini‡*

*Department of Field Crops, Faculty of Agriculture, University of Cukurova, Adana, Turkey; †Istituto Sperimentale per la Cerealicoltura, S. Angelo Lodigiano (LO), Italy; and ‡Max-Planck-Institut für Züchtungsforschung, Köln, Germany

Western agriculture and its most important crop plants are thought to have originated about 10,000 years ago in the Fertile Crescent, a geographical region extending from modern-day Israel, Jordan, Lebanon, and western Syria into southeastern Turkey and along the Tigris and Euphrates rivers into Iraq and Iran (Smith 1995; Bar-Yosef 1998; Diamond 1998; Moore, Hillman, and Legge 2000; Zohary and Hopf 2000; Gopher, Abbo, and Lev-Yadun 2002). Two traditional lines of evidence support that view. First, the geographical distributions of wild progenitors of modern cereal species, among them wild wheats (*Triticum urartu*, *T. boeoticum*, *T. dicoccoides*, *Aegilops tauschii*), wild barley (*Hordeum spontaneum*), and wild rye (*S. vavilovii*), intersect in this region (Nesbitt and Samuel 1996; Moore, Hillman, and Legge 2000; Zohary and Hopf 2000; Gopher, Abbo, and Lev-Yadun 2002). Second, seeds of the wild species occur in early archaeological sites of the region, followed in radiocarbon age and stratigraphic succession by the remains of domesticated forms (Moore, Hillman, and Legge 2000; Zohary and Hopf 2000; Gopher, Abbo, and Lev-Yadun 2002). Recently, molecular evolutionary studies have also begun to weigh heavily on this issue. Genetic identification of the natural stands from which wild crops were domesticated addresses the question of where specifically within the Fertile Crescent humans invented agriculture. The approach involves comparing wild and domesticated populations using molecular markers, which give genome-wide estimates of genetic similarity (Heun et al. 1997; Badr et al. 2000; Martin and Salamini 2000). One of the most promising of these techniques is amplified fragment length polymorphism (AFLP), a polymerase chain reaction (PCR)-based procedure that resolves radioactively labeled electrophoretic bands (polymorphic loci) on sequencing gels.

Using AFLPs, the site of domestication of einkorn—a diploid wheat—was identified from the analysis of 288 AFLP marker loci (Heun et al. 1997). Those results indicated that wild populations from the Karacadag Mountains of southeastern Turkey are more similar to domesticated einkorn than other wild populations are (Heun et al. 1997). Archaeobotanical remains at early settlements near Karacadag, including Cafer Höyük (de Moulins 1993), Cayönü (van Zeist and de Roller 1991–2), Nevali Cori (Pasternak 1998), and Abu Hureyra (de Moulins 2000; Hillman 2000), provided independent evidence for the domestication of einkorn near the Karacadag Range. The publication of the einkorn data (Heun et al. 1997) renewed the debate on the origin of Near East agriculture. Lev-Yadun, Gopher, and Abbo (2000), summarizing the distributions of several cereal and other crop progenitors, reported that these intersect in a small region of southeastern Turkey, circumscribing a small core area that includes Karacadag. Here we address the question of whether the core area was also the place of origin of other additional founder crops of the Fertile Crescent agriculture, using AFLP comparisons at 204 loci from 43 domesticated lines and 99 wild populations of tetraploid wheats—progenitors of modern hexaploid wheats—sampled from primary habitats at known locations.

Domesticated emmer wheat, *T. dicoccum*, has an AABB genome and hulled seeds; a free-threshing form (one that releases seeds during threshing) exists that is called hard wheat (*T. durum*). These two domesticated forms have a nonbrittle rachis (the ear releases seed but stays intact during threshing), in contrast to the progenitor, *T. dicoccoides* (wild emmer), the ears of which fall apart at maturity and thus cannot be threshed. Emmer was the most important crop in the Fertile Crescent until the early Bronze Age (Zohary and Hopf 2000), and domesticated forms are present at several early Neolithic archaeological sites. van Zeist and Bakker-Heeres (1982, 1985) report the presence of domesticated emmer in the lowest excavated level of Tell Aswad, dated 10,800 BP (years before present), but suggest that the plant was introduced from elsewhere. Domesticated emmer archaeological remains (de Moulins 2000) are present, but not common, in layers of Abu Hureyra 2 dating 10,400 BP onward. They are preceded at Abu Hureyra 1 by wild *T. dicoccoides* remains (Hillman 2000). Emmer remains from Cayönü dating from 10,600 BP onward (van Zeist and de Roller 1991–2) suggest a diffuse cultivation of emmer during that time. Pasternak (1998) describes contemporary-like domesticated grains and spikelet forks of emmer at Nevali Cori. In later Pre-Pottery Neolithic B settlements (tables 2 and 14 in Nesbitt and Samuel, 1996, and Helmer et al. 1998, respectively), domesticated emmer is constant and abundant in presence. The dates reported here are calibrated years (BP), that is, they refer to ^{14}C dates that were transformed into calendar years of the absolute dendrochronological record using the data provided by Zohary and Hopf (2000, p. 14) and by Moore, Hillman, and Legge (2000, pp. 130–131) and were cross-checked for consistency with the data of Gopher, Abbo, and Lev-Yadun (2002) and Maier (1996).

Wild emmer, *T. dicoccoides*, hybridizes with domesticated tetraploid wheats, and the hybrids are fertile. The species has brittle ears that shatter (disarticulate) at

Address for correspondence and reprints: F. Salamini, Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné-Weg 10, 50829 Köln, Germany. E-mail: salamini@mpiz-koeln.mpg.de.

Mol. Biol. Evol. 19(10):1797–1801. 2002

maturity into individual spikelets bearing relatively large seeds. It rarely colonizes secondary habitats. In primary habitats, two morphologically distinguishable types are present (Poyarkova 1988). The geographical distribution reported by Zohary and Hopf (2000; p. 45) includes the western Fertile Crescent, its central part in southeastern Turkey, and areas in eastern Iran and Iraq. Johnson (1975) reported that the species is progressively substituted in the transect from southeastern Turkey into Iran-Iraq by the wild tetraploid wheat *T. araraticum*. But in the same areas, occasional *T. dicoccoides* populations are reported to be present among stands of *T. araraticum* (Tanaka and Ishii 1973). This introduces a problem: *T. araraticum* has an AAGG genome and does not produce fertile progeny with *T. dicoccoides* (Maan 1973), but the two species are phenotypically indistinguishable. When sampling *T. dicoccoides* accessions from several gene banks, we rarely received lines collected in Iran or Iraq. This supports Johnson's (1975) conclusion: ''A question is whether authentic *T. dicoccoides* occurs in that area . . . All the tetraploids collected in the Karacadag, in south eastern Anatolia, Lebanon and Israel were *T. dicoccoides*. All of the tetraploids from Transcaucasia and all of those collected in Iraq and Iran, except two, showed the typical *T. araraticum* protein electrophoretic pattern.'' Interestingly, vigorous stands of *T. dicoccoides* grow on the basaltic rocky slopes of the Karacadag Mountains in southeastern Turkey (Harlan and Zohary 1966; Johnson 1975), very close to the site of the origin of einkorn wheat (Heun et al. 1997).

To resolve the relationships of domesticated tetraploid wheats with their wild relatives, we carried out the molecular fingerprinting of wild and domesticated tetraploid lines based on AFLP marker data at 204 loci. The results indicate clearly that domesticated AABB wheats are most closely related to wild populations sampled in southeastern Turkey (fig. 1*A*). For this work, we have used Johnson's *T. dicoccoides* collection because (1) he sampled the collection directly, (2) he had resolved the problematic distinction between *T. dicoccoides* and *T. araraticum* using protein electrophoresis, and (3) a published map (Johnson 1975) indicated exactly where the lines had been sampled, wherein locations corresponded to the United States Department of Agriculture, Agricultural Research Service, National Small Grains Collection (Aberdeen), passport data. Nineteen wild emmer lines from Karacadag populations were included in the analysis. Fifteen out of the 19 lines had topologies consistent with their close genetic relationships to domesticated emmer (fig. 1*A*). These fifteen lines are included in groups III and IV (fig. 1*A*) to which a few other lines belong that were also sampled in southeastern Turkey ~200 km west and east from Karacadag. Of all other wild emmer lines considered, only a single Syrian line clustered together with Karacadag lines in group III. Wild populations sampled in Israel clustered mainly in groups V, VII, and VIII, with group V being more closely related than are groups VII and VIII to southeastern Turkey populations. When AFLP allelic frequencies in populations sampled within different regions of the area—Lebanon, Israel, Syria, Jordan, and Turkey—were considered (fig. 1*B*), the southeastern Turkey populations were also found to be more similar to domesticated tetraploid wheats than were any other wild populations sampled. A *T. dicoccoides* accession from a secondary habitat at Izmir, Turkey, was also closely related to cultivated emmer, as was the line Dic 196 collected at Bakhtaran, Iran (labelled ''In'' in fig. 1*A*), which clustered with hulled emmer landraces. Such cases likely represent wild lines growing in secondary habitats, that is, germplasm which was displaced by humans during the early spread of agriculture, followed by subsequent naturalization of these lines outside their primary habitats.

Figure 1*C* shows a neighbor-joining tree of Dice distances considering the tetraploid lines as in figure 1*A*, to which *T. urartu*, *T. boeoticum*, and *T. araraticum* lines were added. The latter three lines are shown to be genetically distinct from all other tetraploids, supporting the species assignment of Johnson (1975) for all *T. dicoccoides* and *T. dicoccum* lines studied.

As for other wheats, the wild and domesticated tetraploid lines differed most dramatically in rachis fragility and seed weight (table 1). In this respect, wild wheats sampled in southeastern Turkey (groups III and IV) had domestication-related traits totally indistinguishable from those of other wild *T. dicoccoides* lines.

In figure 1*A*, all lines of hulled emmer were included in group C1 and all *T. durum* free-threshing genotypes in group C2, indicating a single origin of both. Moreover, the two groups emerged quite early from Turkish wild *T. dicoccoides*. This supports the early appearance of domesticated tetraploid naked wheats in excavated sites and helps to explain the otherwise puzzling report of domesticated grains belonging to both tetraploid and hexaploid free-threshing forms at the excavated sites of Abu Hureyra 2 (de Moulins 2000) and at other early Neolithic sites (Zohary and Hopf 2000).

The findings reported here localize the origin of tetraploid wheat domestication to southeastern Turkey and thus lend strong support to the emerging view that agriculture originated in an extremely small core area in the Near East, specifically in southeastern Turkey near the Tigris and Euphrates rivers (Heun et al. 1997; Lev-Yadun, Gopher, and Abbo 2000; Gopher, Abbo, and Lev-Yadun 2002). This site of emmer domestication lies within the region already known to host wild progenitors of pea, chickpea, lentils, and einkorn, all of which founder crops were present in the ''package'' of crops that rapidly emerged during the origin of agriculture in the Fertile Crescent some 10,000 years ago (Gopher, Abbo, and Lev-Yadun 2002). But an exception to this rule is the case of barley, which appears to have been domesticated in the Jordan Valley, on the western edge of the Fertile Crescent (Badr et al. 2000). This discrepancy is accounted for by the possibility that in early settlements of the Jordan Valley, wild barley, rather than wild wheats, was preferentially harvested from wild stands (Willcox 1995; Nesbitt and Samuel 1996). Thus, barley may have been domesticated in the Jordan valley only after local communities had imported a technology developed elsewhere (the core area in southeastern Tur-

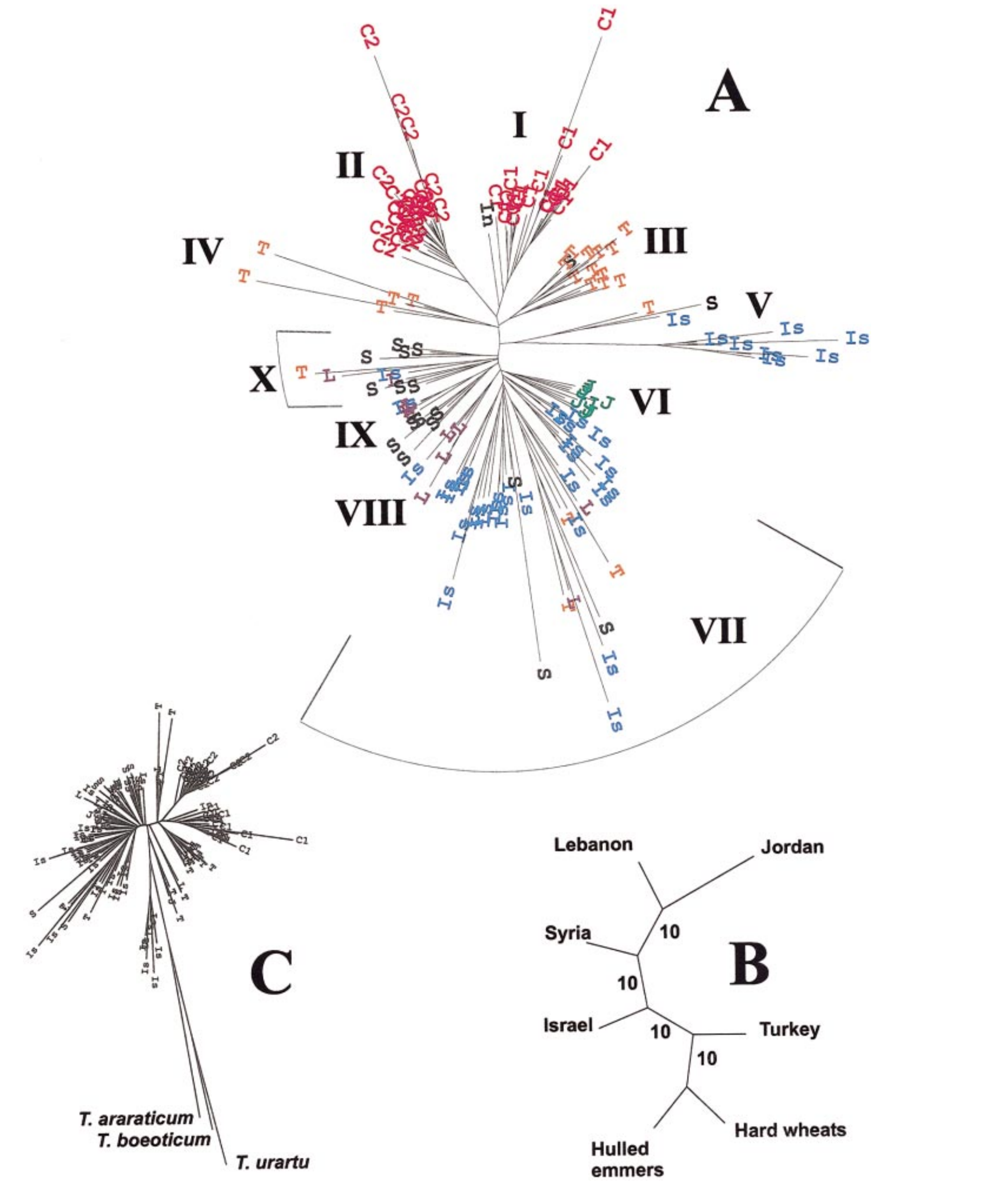

FIG. 1.—Genetic relationships of hulled emmer, free-threshing hard wheat, and wild emmer lines. *A,* AFLP phylogeny of 43 cultivated tetraploid wheats (19 hulled land races of emmer [*T. dicoccum*], designated C1, and 24 free-threshing hard wheat [*T. durum*] varieties, designated C2) and 99 wild emmer lines from southeastern Turkey (T; 22), Israel (Is; 37), Jordan (J; 8), Lebanon (L; 13), Syria (S; 18), and Iran (In; 1) (see also table 1). The neighbor-joining tree (Saitou and Nei 1987) of Dice (1945) genetic distances is shown. Cultivated emmer lines were from Turkey, Rumania, Iran, India, Germany, and Italy, the hard wheat lines were from France, Palestine, Italy, Tunisia, Jordan, Cyprus, Spain, Syria, Greece, Ukraine, Tajikistan, and Mexico. All were grown during spring-summer 2001 at Cologne together with wild lines of *T. urartu, T. boeoticum,* and *T. araraticum.* In total, 204 polymorphic AFLP loci were scored for presence or absence of an AFLP fragment in each of the 142 lines (all details available upon request). Within Triticeae, 90% of AFLP bands segregating in experimental populations define a Mendelian locus (Castiglioni et al. 1998; Taenzler et al. 2002). *B,* Similarities of cultivated tetraploid wheats to wild emmer populations sampled in different regions. Using programs of the PHYLIP package (1993), 10 independent trees were constructed as described (Heun et al. 1997; Badr et al. 2000) using CONTML (topology shown; Felsenstein 1981) and distance matrix methods (Fitch and Margoliash 1967; Saitou and Nei 1987) employing various measures of genetic distance (Cavalli-Sforza and Edwards 1967; Nei 1972; Wright 1978; Reynolds, Weir, and Cockerham 1983; Rohlf 1993) calculated from AFLP allele frequencies between lines from geographic regions indicated. All methods gave the same topology (indicated at branches). *C,* Neighbor-joining tree of Dice distances for tetraploid wheats as in *A,* but including the same 204 AFLPs for *T. urartu*, *T. boeoticum*, and *T. araraticum*, which are seen here to be genetically very distinct from the wild and cultivated tetraploid lines shown in *A,* consistent with Johnson's (1975) species assignments for the *T. dicoccoides* or *T. dicoccum* lines studied.

Table 1
Average Values (± SD) per Group of Three Characters with Diagnostic Value in Discriminating Wild from Domesticated Germplasm. The Groups of Lines were Assorted Based on AFLP Molecular Fingerprinting. Values Reported are Derived from Five Different Samples

Group[a]	No. of lines considered	Leaf length (cm)	Rachis fragility[b]	Seed weight (mg)
C1 (Domesticated; hulled emmer)	19	20.0 ± 2.8	5.0 ± 0.0	46.3 ± 7.9
II C2 (Domesticated; hard wheats)	24	21.8 ± 3.1	5.0 ± 0.0	46.8 ± 8.6
III T (Wild, mainly from Turkey)	13	13.3 ± 2.9	1.9 ± 1.0	18.6 ± 3.6
IV T (Wild, from Turkey)	5	16.5 ± 1.2	1.8 ± 1.1	18.0 ± 3.3
V Is (Wild, mainly from Israel)	10	14.1 ± 3.1	1.6 ± 1.0	25.7 ± 7.8
VI J (Wild, mainly from Jordan)	8	12.1 ± 2.5	1.0 ± 0.0	23.7 ± 6.5
VII Is (Wild, mainly from Israel)	35	13.4 ± 1.8	1.9 ± 1.0	24.2 ± 3.5
VIII L (Wild, mainly from Lebanon)	5	11.0 ± 2.2	1.0 ± 0.0	21.8 ± 1.5
IX S (Wild, mainly from Syria)	11	12.1 ± 3.6	1.7 ± 1.0	19.1 ± 3.4
X S (Wild, mainly from Syria)	11	11.3 ± 1.8	1.4 ± 0.8	20.8 ± 4.3

[a] Indicated as in Fig. 1A.
[b] 1 brittle; 5 tough.

key) that allowed them to deliberately cultivate wild crop plants.

LITERATURE CITED

Badr, A., K. Müller, R. Schafer-Pregl, H. El Rabey, Effgen, S., H. H. Ibrahim, C. Pozzi, W. Rohde, and F. Salamini. 2000. On the origin and domestication history of barley. Mol. Biol. Evol. **17**:499–510.

Bar-Yosef, O. 1998. The Natufian Culture in the Levant, threshold of the origin of agriculture. Evol. Anthropol. **6**: 159–177.

Castiglioni, P., C. Pozzi, M. Heun, K. J. Müller, W. Rohde, and F. Salamini. 1998. An AFLP-based procedure for the efficient mapping of mutants and DNA probes in barley. Genetics **149**:2039–2056.

Cavalli-Sforza, L. L., and A. W. F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution **32**:550–570.

de Moulins, D. 1993. Les restes de plantes carbonisées Cafer Höyük. Cah. l'Euphrate **7**:191.

de Moulins, D. 2000. Abu Hureyra 2: plant remains from the Neolithic. Pp. 399–422 *in* A. M. T. Moore, G. C. Hillman, and A. J. Legge, eds. Village on the Euphrates: from foraging to farming at Abu Hureyra. Oxford University Press, New York.

Diamond, J. 1998. Guns, germs and steel. A short history of everybody for the last 13,000 years. Vintage, Random House UK Limited.

Dice, L. R. 1945. Measures of the amount of ecologic association between species. Ecology **26**:297–302.

Felsenstein, J. 1981. Evolutionary trees from gene frequencies and quantitative characters: finding maximum likelihood estimates. Evolution **35**:1229–1242.

Fitch, W. M., and E. Margoliash. 1967. Construction of phylogenetic trees. Science **155**:279–284.

Gopher, A., S. Abbo, and S. Lev-Yadun. 2002. The ''when'', the ''where'' and the ''why'' of the Neolithic revolution in the Levant. Doc. Praehist. **28**:1–14.

Harlan, J. R., and D. Zohary. 1966. Distribution of wild wheats and barley. Science **153**:1074–1080.

Helmer, D., V. Roitel, M. Sana, and G. Willcox. 1998. Interpretations environmentales des donnees archeozoologiques et archeobotaniques en Syrie du Nord de 16,000 BP a 7,000 BP, et les debuts de la domestication des plantes et des animaux. Pp. 9–34 *in* M. Forin and O. Aurench, eds. Natural spece, inhabited space in Northern Syria. 10th Millennium B.C. Bulletin 33.

Heun, M., R. Schaefer-Pregl, D. Klawan, R. Castagna, M. Accerbi, B. Borghi, and F. Salamini. 1997. Site of einkorn wheat domestication identified by DNA fingerprinting. Science **278**:1312–1314.

Hillman, G. C. 2000. The plant food economy of Abu Hureyra 1 and 2: Abu Hureyra 1: the Epipaleolithic. Pp. 327–398 *in* A. M. T. Moore, G. C. Hillman, and A. J. Legge, eds. Village on the Euphrates: from foraging to farming at Abu Hureyra. Oxford University Press, New York.

Johnson, B. L. 1975. Identification of the apparent B-genome donor of wheat. Can. J. Genet. Cytol. **17**:21–39.

Lev-Yadun, S., A. Gopher, and S. Abbo. 2000. The cradle of agriculture. Science **288**:1602–2603.

Maan, S. S. 1973. Cytoplasmic and cotogenetic relationships among tetraploid *Triticum* species. Euphytica **22**:287–300.

Maier, U. 1996. Morphological studies of free-threshing wheat ears from a Neolithic site in southwest Germany, and the history of naked wheats. Veg. Hist. Archaeobot. **5**:39–55.

Martin, W., and F. Salamini. 2000. A meeting at the gene. Biodiversity and natural history. EMBO Rep. **1**:208–210.

Moore, A. M. T., G. C. Hillman, and A. J. Legge. 2000. Village on the Euphrates. Oxford University Press, Oxford, N.Y.

Nei, M. 1972. Genetic distance between populations. Am. Nat. **106**:283–292.

Nesbitt, M., and D. Samuel. 1996. From staple crop to extinction? The archaeology and history of the hulled wheats. Pp. 41–100 *in* S. Padulosi, K. Hammer, and J. Heller, eds. Hulled wheats. Proceedings of the 1st International Workshop on Hulled Wheats. Castelvecchio Pascoli, Italy.

Pasternak, R. 1998. Investigations of botanical remains from Nevali Cori PPNB, Turkey: a short interim report. Pp. 170–176 *in* A. B. Damania, J. Valkoun, G. Willcox, and C. O. Qualset, eds. The origins of agriculture and crop domestication. ICARDA, Aleppo, Syria.

Poyarkova, U. 1988. Morphology, geography and infraspecific taxonomics of *Triticum dicoccoides*, Körn. A retrospective of 80 years of research. Euphytica **38**:11–23.

Reynolds, J. B., B. S. Weir, and C. C. Cockerham. 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. Genetics **105**:767–779.

ROHLF, F. J. 1993. NTSYS-pc. Numerical taxonomy and multivariate analysis system. Applied Biostatistics Inc., New York.

SAITOU, N., and M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**:406–425.

SMITH, B. D. 1995. The emergence of agriculture. Scientific American Library, New York.

TAENZLER, B., R. F. ESPOSTI, P. VACCINO, A. BRANDOLINI, S. EFFGEN, M. HEUN, R. SCHAEFER-PREGL, B. BORGHI, and F. SALAMINI. 2002. A molecular linkage map of einkorn wheat: mapping of storage-protein and soft-glume genes and bread-making quality QTLs. Genetical Res. (in press).

TANAKA, M., and H. ISHII. 1973. Cytogenetic evidence on the specidation of wild tetraploid wheats collected in Iraq, Turkey and Iran. Pp. 115–121. Proc. 4th Int. Wheat Genet. Symp. University of Missouri.

VAN ZEIST, W., and J. A. H. BAKKER-HEERES. 1982. Archaeobotanical studies in the Levant. 1. Neolithic sites in the Damscus basin: Aswad, Ghoraifé, Ramad. Palaeohistoria **24**:165–256.

———. 1985. Archaeobotanical studies in the Levant 4. Bronze ages sites on the north Syrian Euphrates. Palaeohistoria **27**:247–316.

VAN ZEIST, W., and G. J. DE ROLLER. 1991–2. The plant husbandry of aceramic Cayönü, S.E. Turkey. Palaeohistoria **33/34**:65–96.

WILLCOX, G. 1995. Wild and domesticated cereal cultivation: new evidence from early Neolithic sites in the northern Levant and south-eastern Anatolia. ARX World J. Prehistoric Ancient Studies **1**:9–16.

WRIGHT, S. 1978. Evolution and the genetics of populations. In: Variability within and among natural populations 4, Chicago Press, Chicago.

ZOHARY, D., and M. HOPF. 2000. Domestication of plants in the old world: the origin and spread of cultivated plants in West Asia, Europe, and the Nile Valley. Oxford University Press, New York.

WILLIAM MARTIN, reviewing editor

Accepted May 2, 2002